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(54) **Compositions comprising glutathione liposomes**

(57) The invention provides compositions suitable for topical application comprising glutathione liposomes together with at least one emulsifier. Such compositions

are capable of preventing or reducing the effects of free radicals in the skin, in particular the damaging effects of UV radiation.

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Description

The present invention relates to compositions comprising glutathione liposomes. In particular, the invention relates to the use of such compositions to help maintain or restore the skin's natural levels of glutathione, thereby preventing or reducing the harmful effects of UV radiation on the skin. Such compositions find particular use in an after-sun preparation.

The damaging effects of UV radiation on the skin are well documented. Short term effects of exposure to UV radiation include erythema (i.e. sunburn), whilst the long term effects of repeated exposure to UV radiation include skin cancers and premature ageing of the skin.

The formation of free radicals in the skin is believed to be a primary cause of the harmful effects of sunlight upon the skin. Whilst the skin itself contains antioxidants or free radical scavengers, the natural levels of these deplete rapidly on exposure to UV radiation, thus significantly reducing the body's own defence against damage from free radicals.

Amongst the free radical scavengers naturally present in the skin is glutathione. However, exposure to UV radiation is believed to be capable of reducing the skin's natural levels of glutathione in as little as 4 minutes, thereby reducing the body's natural defence against the harmful effects of free radicals. There thus exists a need for formulations which can be topically applied to the skin and which serve to replenish the skin's natural levels of glutathione, thereby preventing or reducing damage to the skin resulting from exposure to UV radiation.

Glutathione itself is unable to penetrate through the stratum corneum and applied topically to the skin in a simple oil-in-water emulsion or in the form of a gel preparation has been found to be ineffective in inhibiting UVB induced skin erythema (Int. J. Cos. Sci. 17:91-103, 1995). Whilst glutathione in the form of a liposomal gel preparation has been suggested to be effective in inhibiting UVB skin erythema, there still exists a need for formulations which are more effective in preventing or reducing the harmful effects of UV radiation, in particular formulations which can provide rapid relief from the effects of free radicals.

It has now surprisingly been found that an emulsion comprising glutathione liposomes provides a particularly rapid delivery of glutathione into the skin, thus serving to rapidly replenish and maintain the skin's natural levels of glutathione and thereby provide rapid relief from the effects of UV radiation.

Viewed from one aspect, the invention thus provides a composition suitable for topical application comprising glutathione liposomes, preferably in an amount of from 0.5 to 10% by weight, e.g. from 1 to 5% by weight, together with at least one emulsifier, preferably in an amount of from 0.1 to 10% by weight, e.g. from 1 to 5% by weight.

The glutathione for use in accordance with the invention is preferably present in the form of reduced glutathione. Derivatives of glutathione, including its salts and esters, may however be used.

Glutathione liposomes suitable for use in accordance with the invention preferably comprise an aqueous solution containing reduced glutathione enclosed within a multilamellar liposomal matrix. Preferred liposomes are those comprising from 0.5 to 5.0% by weight, preferably from 1.5 to 2.3% by weight phospholipids which facilitate liposome penetration. Preferably, the liposome will range in size from 50 to 500nm, e.g. up to about 200nm. Suitable phospholipids for making the liposomes include phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol which may be present in amounts of from 18 to 26% by weight, from 16 to 21% by weight and from 12 to 18% by weight respectively. The liposomal matrix may further contain preservatives to prevent spoilage by microorganisms. The final concentration of reduced glutathione present in the liposomal matrix is conveniently from 0.01 to 1.0% by weight, preferably 0.1% by weight.

Liposomes for use in accordance with the invention can be prepared according to methods known in the art. For example, these can be prepared by dissolving at least one liposome forming compound in a suitable solvent.

Suitable emulsifiers include non-ionic, cationic and anionic emulsifiers known in the art. Non-ionic emulsifiers such as cetyl alcohol, cetearyl alcohol, mono-, di- and triglycerides of fatty acids, ethoxylated fatty alcohols or ethoxylated fatty acids may conveniently be present in an amount of from 0.05 to 10% by weight. Anionic emulsifiers such as sodium, potassium or amine salts of fatty acids, of cetyl phosphates or of an acrylates/alkyl acrylate cross polymer may be present in an amount of from 0.01 to 10% by weight.

Other components which may be present in the compositions of the invention include those known to provide additional protection to the skin against the effects of oxidant species such as superoxide anion, singlet oxygen and peroxy radicals formed during photochemical reactions. Such components include vitamins A, C and E and their acid esters, superoxide dismutase (SOD) and reduced glutathione and may be present in either a free or a liposomal form, typically at concentrations of from 0.001 to 10% by weight. Conveniently, these may be present within the same liposome as the glutathione.

The compositions of the invention may conveniently contain one or more agents which serve to soothe the skin or ameliorate the burning effects of the sun. Examples of suitable agents include calamine, camphor, camomile extract, gamma-linolenic acid, hamamelis, hydrocortisone, allantoin, aloe vera and urea. Such ingredients may be present at concentrations of from 0.01 to 10% by weight.

In a preferred embodiment of the invention, particular antioxidant/liposome combinations can be formulated to

provide the desired level and duration of antioxidant activity, e.g. to provide an instant release of anti-oxidants to the skin.

The compositions of the invention preferably further comprise at least one pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable" is used to define any agent suitable for topical administration and generally recognised as safe for topical application to the skin.

The pharmaceutically acceptable carrier typically comprises from 0.1 to 99 % by weight of the compositions of the invention, preferably from 80 to 90 % by weight.

Other components which may be present in the compositions of the invention include oils or esters, thickening and/or stabilising agents, moisturisers, preservatives and/or chelating agents, antioxidants, fragrance and colourings.

Oils or esters may be present as emollients to provide an acceptable skin-feel. Examples of oils which may be used include mineral oil, plant oils such as sweet almond oil, avocado oil and jojoba oil, each of which may conveniently be present at concentrations of from 0.5 to 20% by weight. Examples of suitable esters include those formed from a fatty acid and fatty alcohol. Preferred esters include isopropyl myristate, cetearyl octanoate and octyl cocoate. Oils and/or esters may conveniently be present in amounts of from 0.5 to 20% by weight.

Examples of suitable thickening/stabilising agents include the carbomer type anionic water phase thickening, gelling and structuring agents, mineral thickeners such as magnesium aluminium silicate and natural gums such as xanthan gum, which may be present in concentrations of from 0.01 to 10% by weight. Other suitable thickening/stabilising agents include oil phase thickening and structuring agents such as fats and fatty acids, e.g. stearic acid; natural and synthetic waxes and microcrystalline hydrocarbon waxes conveniently present at concentrations of from 0.5 to 20% by weight.

A highly preferred component of the compositions of the invention is at least one moisturiser. A variety of moisturisers may be present, conveniently in an amount of from 0.1 to 20% by weight. Humectant or occlusive moisturising systems which encourage the retention of water within the stratum corneum are preferred. A suitable humectant system may include glycerol, sorbitol or propylene glycol or other constituents equivalent to those found naturally in the skin, such as pyrrolidone carboxylic acid. Suitable occlusive systems include silicones, petrolatum or mineral oil.

Conveniently, the compositions of the invention will include at least one preservative agent or chelating agent designed to control the growth of microorganisms within the product or to control the presence of free metal ions. Examples of suitable substances include potassium sorbate, methylidibromoglutaronitrile, methyl, ethyl, propyl and butyl esters of p-aminobenzoic acid and their metal salts, phenoxyethanol, propylene glycol and disodium edetate, conveniently present in an amount of from 0.005 to 5% by weight.

The compositions in accordance with the invention may further comprise one or more antioxidants, conveniently present in an amount of from 0.005 to 1% by weight, and designed to prevent the oxidative degradation of the product or its constituents. Examples of suitable antioxidants include butylatedhydroxytoluene (BHT) or butylatedhydroxyanisole (BHA).

The compositions in accordance with the invention may be formulated in any conventional form, including oils, emulsions, milks, lotions, creams, mousses, sticks, ointments, gels etc. which may be applied topically to the skin by normal application or by spraying.

The compositions in accordance with the invention are of particular use in helping to maintain or restore the natural levels of glutathione in the skin and thus are effective in preventing or reducing the effects of free radicals on the skin. Such compositions are particularly effective in treating conditions which result from the presence of free radicals in the skin, such as inflammatory conditions caused by exposure to UV radiation, e.g. skin erythema. The compositions are also effective in inhibiting photoaging resulting from long-term exposure of the skin to UV radiation. Viewed from a further aspect, the invention thus provides compositions as hereinbefore defined for use in the prevention or treatment of conditions resulting from the presence of free radicals in the skin.

Viewed from a yet further aspect the invention provides the use of compositions as hereinbefore defined in the manufacture of a medicament for use in the prevention or treatment of conditions resulting from the presence of free radicals in the skin.

In another aspect the invention provides a method of cosmetic treatment of the human or non-human mammalian body to combat or prevent conditions associated with the presence of free radicals in the skin, said method comprising application to the skin surface of said body an effective amount of a composition as hereinbefore defined.

The compositions of the invention may be applied to the skin on a daily basis as a moisturiser to protect the skin against the harmful effects of UV radiation, e.g. to prevent photoaging of the skin. Alternatively, these may be applied to the skin following prolonged exposure to higher levels of UV radiation, e.g. as an after-sun preparation.

The compositions of the invention are applied to the skin in an effective amount, e.g. in an amount equivalent to 2mg of the product per cm² of skin.

Preferred embodiments of the invention will now be described by way of the following non-limiting examples:

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Example 1 - After-Sun Formulation

	% by weight
<i>Phase A</i>	
Glyceryl Tricaprylate/caprate	5.0
Myristyl Myristate	2.0
Dimethicone	2.0
Cetostearyl Alcohol	2.5
Glyceryl Stearate	0.8
Allantoin	0.2
Cetoth-20	2.5
<i>Phase B</i>	
Magnesium Aluminium Silicate	0.7
Disodium Edetate Dihydrate	0.1
Glycerol	4.0
Preservative	qs
Water	ad 100
<i>Phase C</i>	
Glutathione Liposome (containing 0.1% reduced glutathione)	5.0
Monthyl Lactate	1.0
Fragrance	qs

The ingredients of Phase A are mixed and heated to 75°C. The ingredients of Phase B are mixed and heated to 75°C. Phase A and Phase B are then mixed until homogeneous and allowed to cool. When the mixture has cooled to below 40°C, the ingredients of Phase C are added and mixed slowly. The resultant mixture is allowed to cool to room temperature.

Example 2 - After-Sun Formulation

	% by weight
<i>Phase A</i>	
Glyceryl Tricaprylate/caprate	2.0
Isopropyl Myristate	2.0
Dimethicone	0.1
Cetearyl Octanoate	2.0
Glyceryl Stearate SE	2.5
Cetearyl Alcohol	2.0
Sodium Lauryl Sulphate	0.02
Allantoin	0.2
<i>Phase B</i>	
Carbomer	0.2
Triethanolamine	0.5
Disodium Edetate Dihydrate	0.1
Propylene Glycol	3.0
Disodium Edetate Dihydrate	0.05

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(continued)

	% by weight
<i>Phase B</i>	
Preservative	qs
Water	ad 100
<i>Phase C</i>	
Glutathione Liposome (containing 0.1% reduced glutathione)	5.0
Fragrance	qs

The formulation is prepared analogously to that described in Example 1.

Example 3 - efficacy of glutathione liposome formulation

The depth of glutathione penetration into the skin was determined by comparing two different galenic forms: free glutathione in solution (FG) and glutathione encapsulated in a liposome (LG). Since glutathione is a highly reactive free radical scavenger, its presence even in small quantities can be detected by spectroscopic methods.

Protocol:

Gluthione preparations are applied to the skin, and tape strippings are taken after some fixed interval of time. The tape strippings are assayed for anti-free radical activity by DPPH (1,1 Diphenyl-2-picrylhydrazyl) at $8.76 \times 10^{-3} \%$ (w/v) in methanol) colouration/ discolouration tests (Kim et al. Proc. 17th IFSCC Congress (Yokohama), Vol. 1: 3-14, 1994). The number of strippings necessary to obtain total loss of activity is a measure of the depth of penetration (Clark, J. Soc. Cosmet. Chem. 43: 219-227, 1992; Rougier A et al., J. Pharm. Sci. 76: 451-454, 1987; Thibault et al., Proc. 17th IFSCC Congress (Yokohama), Vol. 1: 245-250, 1992).

Method:

10 healthy male and female volunteers of 22 to 45 years of age participated in the study. Tape strippings were carried out on the volar forearm of each volunteer with the aid of BLENDERM® adhesive tape (3M Santé).

Light absorption measurements were carried out in a KONTRON UV-Vis spectrometer at 517nm.

Solutions tested:

Reduced glutathione in water at 0.1% concentration (FG).

Glutathione in liposome at equal concentration (LG).

On the arms of each volunteer, the 3 test sites were determined in a random way: one site for application of water (negative control), one for application of free glutathione (FG) and one for application of liposome encapsulated glutathione (LG). The sites were softly washed with a cloth dipped into ethanol to remove lipids, then allowed to dry and equilibrate for 2 minutes before product application.

5 tape strippings were carried out on the control sites after $2\mu\text{l}/\text{cm}^2$ application of distilled water and a light massage. 30 minutes later, the strips were then cut into halves (3.5cm of diameter) onto which was placed a plastic bell of 3cm diameter at the bottom and having a small aperture at the top. Then 1ml of ethanol/water (85/15) solution was introduced into the bell and 500 μl of DPPH solution in methanol were added. After 10 minutes the reaction was stopped by inverting the bell and emptying its content into a microcell (1ml). The change in colour was measured on a spectrophotometer at 517nm.

Each test solution was then applied to the designated sites ($2\mu\text{l}/\text{cm}^2$) by slight massaging and left to dry for 30 minutes. 5 successive strips were then taken and contacted with the DPPH solutions. Colour changes were recorded as described above.

Results:

The table below shows the average depth of penetration as revealed by the presence of DPPH reactive substances

on the successive strips.

The negative controls indicate the absence of detectable glutathione in untreated skin.

As to the free glutathione preparation (FG), the absence of significant penetration is noted: the product remains concentrated within the first two strips.

The liposome encapsulated glutathione (LG), on the other hand, is carried into the deeper layers of the stratum corneum. Statistical analysis of the 10 values showed the difference to be significant at the 95% confidence level.

Table 1

Tape strips containing residual scavenger activity			
Person	Control	FG	LG
1	0	1	2
2	0	1	2
3	0	2	2
4	0	1	2
5	0	1	3
6	0	1	3
7	0	2	1
8	0	1	1
9	0	2	1
10	0	1	2
Mean value	0	1.3	1.90
SEM	0	0.48	0.74

Claims

1. A composition for topical application comprising glutathione liposomes, together with at least one emulsifier.
2. A composition as claimed in claim 1 wherein said glutathione liposomes are present in an amount of from 0.5 to 10% by weight, preferably from 1.0 to 5% by weight.
3. A composition as claimed in claim 1 or claim 2 wherein said glutathione liposomes comprise an aqueous solution comprising reduced glutathione enclosed within a multilamellar liposomal matrix.
4. A composition as claimed in any one of claims 1 to 3 wherein the concentration of reduced glutathione in the liposomal matrix is from 0.01 to 1.0% by weight, preferably 0.1% by weight.
5. A composition as claimed in any preceding claim wherein said emulsifier is present in an amount of from 0.1 to 10% by weight, preferably from 1.0 to 5% by weight.
6. A composition as claimed in any preceding claim wherein said emulsifier is selected from non-ionic, cationic and anionic emulsifiers, and mixtures thereof.
7. A composition as claimed in claim 6 wherein said non-ionic emulsifier is selected from cetyl alcohol, cetearyl alcohol, mono-, di- and triglycerides of fatty acids, ethoxylated fatty alcohols, ethoxylated fatty acids and mixtures thereof.
8. A composition as claimed in claim 6 wherein said anionic emulsifier is selected from sodium, potassium and amine salts of fatty acids, of cetyl phosphates or of an acrylates/alkyl acrylate cross polymer, and mixtures thereof.
9. A composition as claimed in any preceding claim further comprising one or more compounds selected from vitamins A, C and E and their acid esters, superoxide dismutase and reduced glutathione, each of which may be present in free or liposomal form.
10. A composition as claimed in any preceding claim further comprising one or more compounds selected from cala-

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mine, camphor, camomile extract, gamma-linolenic acid, hammamelis, hydrocortisone, allantoin, aloe vera and urea.

- 5
11. A composition as claimed in any preceding claim further comprising at least one moisturiser.
12. A composition as claimed in any preceding claim for use in the prevention or treatment of conditions resulting from the presence of free radicals in the human or non-human mammalian skin.
- 10
13. Use of a composition as claimed in any one of claims 1 to 11 in the manufacture of a medicament for use in the prevention or treatment of conditions resulting from the presence of free radicals in the human or non-human mammalian skin.
14. A method of cosmetic treatment of the human or non-human mammalian body to combat or prevent conditions associated with the presence of free radicals in the skin, said method comprising application to the skin surface of said body of an effective amount of a composition as claimed in any one of claims 1 to 11.
- 15
15. A composition as claimed in any one of claims 1 to 11 for use in protecting the skin of a human or non-human mammalian body from the harmful effects of UV radiation.
- 20
16. Use of a composition as claimed in any one of claims 1 to 11 in the manufacture of a medicament for use in protecting the skin of a human or non-human mammalian body from the harmful effects of UV radiation.
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EUROPEAN SEARCH REPORT

Application Number
EP 97 30 7500

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate of relevant passages	Relevant to claim:	CLASSIFICATION OF THE APPLICATION (Int.Cl. 6)
X	FR 2 609 393 A (LABORATOIRES SEROBIOLOGIQUES) * claims 1-3, 5, 6 * * examples 1, 2, 5, 9, 14 *	1-4, 6-8, 10	A61K7/00 A61K7/48
X	WO 95 34280 A (FROSTER & GAMBLE) * claims 1, 2 * * page 5, line 30-34 * * page 6, line 3-26 * * page 10, line 1-6 * * page 12, line 26 - page 13, line 5 * * page 14, line 21-25 * * page 15, line 1-8 *	1, 2, 6-10, 12-16	
X	FR 2 714 600 A (L'OREAL) * claims 1, 3 * * page 8, line 18 - page 9, line 10 * * page 9, line 40-44 * * page 10, line 35-38 * * page 12, line 45 - page 13, line 14 *	1, 6-9, 11, 13-16	
			TECHNICAL FIELDS SEARCHED (Int.Cl. 6)
			A61K
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 6 January 1998	Examiner Peeters, J.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure F : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

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